

Sub B1

1. (once amended) A method of counting a single copy of a target species immobilized on a substrate, said method comprising:

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(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, and

(ii) resolving said fluorescence from said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

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4. (once amended) The method according to claim 1, wherein said target species has a first quantum dot and a second quantum dot attached thereto and said first quantum dot is distinguishable from said second quantum dot.

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10. (once amended) The method according to claim 1, wherein said first quantum dot is attached to a targeting moiety for said target species, said targeting moiety being a member selected from the group consisting of antibodies, aptamers, proteins, streptavidin, nucleic acids and biotin.

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22. (once amended) The method according to claim 19, wherein said alignment moiety identifies the position of one or more target moiety-affinity complex.

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25. (once amended) A computer-readable medium encoded with a data set comprising data acquired by the method of claim 1.

28. (once amended) A computer-readable medium encoded with a database comprising two or more data sets according to claim 25, wherein said database is in a searchable format.

29. (once amended) A method of counting a single copy of a target species in solution, said method comprising

(i) detecting a single copy of said target species by detecting essentially simultaneously fluorescence emitted by a first quantum dot of a first color attached to said single copy and a second quantum dot of a second color attached to said single copy, wherein said first color and said second color are distinguishably different colors, and

(ii) resolving said fluorescence emitted by said first quantum dot of a first color attached to said single copy and said second quantum dot of a second color attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

30. (once amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate with spacing between each member of said population, said method comprising:

(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate, wherein said detecting is performed with a detecting means having a resolution that is higher than said spacing between each member of said population, and

(ii) resolving said fluorescence emitted by said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

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31. (once amended) A method of counting a single copy of a target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(i) detecting a single copy of said target species by detecting fluorescence emitted by a quantum dot attached to said single copy, wherein said single copy is bound to an affinity moiety for said target species immobilized on said substrate forming a target-affinity moiety complex, and said detecting is performed with a detecting means having a resolution limited region of interest whereby, less than one target-affinity moiety complex is present within each resolution limited region of interest, and

(ii) resolving said fluorescence emitted by said quantum dot attached to said single copy from fluorescence arising from a quantum dot not attached to said single copy, thereby counting said single copy.

32. (once amended) A method of counting a single copy of a first target species immobilized on a substrate, which species is a member of a population of target species immobilized on said substrate, said method comprising:

(a) defining a first region of interest of said substrate; and

(b) probing said first region of interest for fluorescence emitted by a quantum dot attached to said single copy of said first target species bound to an affinity moiety for said first target species immobilized on said substrate, wherein said probing resolves said fluorescence from said quantum dot from fluorescence arising from other members of said population of target species immobilized on said substrate, thereby counting said first target species.

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33. (once amended) The method according to claim 32, further comprising counting a single copy of a second target species immobilized to said substrate, said method comprising:

(c) defining a second region of interest of said substrate; and

(d) probing said second region of interest for fluorescence emitted by a second quantum dot attached to said single copy of said second target species bound to an affinity moiety for said second target species immobilized on said substrate, wherein said probing resolves said fluorescence from said second quantum dot from fluorescence arising from other members of said population of target species immobilized on said substrate, thereby counting said second target species.

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37. (once amended) A method for counting multiple target species immobilized on a substrate, which species are members of a population of target species immobilized on said substrate, said method comprising:

(a) defining multiple regions of interest on said substrate; and

(b) probing said multiple regions of interest for fluorescence emitted by a quantum dot attached to a single copy of said target species bound to an affinity moiety for said target species immobilized within a region of interest of said substrate, wherein said probing resolves fluorescence from said quantum dot from other members of said population and from each other, thereby counting multiple target species.

38. (once amended) A method for determining whether a target species within a region of interest on a substrate is quantifiable by a technique selected from the group consisting of single target counting and ensemble counting, said method comprising: